American Journal of Medical Genetics Part A

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Journal:	*American Journal of Medical Genetics: Part A*
Manuscript ID	18-0913.R1
Wiley - Manuscript type:	Clinical Report
Date Submitted by the Author:	n/a
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Keywords:	Acrodysostosis, Pseudohypoparathyroidism, Pseudopseudohypoparathyroidism, Albright Hereditary Osteodystrophy, iPPSD
Search Terms:	Acrodysostosis, Pseudohypoparathyroidism, Pseudopseudohypoparathyroidism, Albright Hereditary Osteodystrophy, iPPSD

Identification of novel pathogenic variants and features in patients with pseudohypoparathyroidism and acrodysostosis, subtypes of the newly classified inactivating PTH/PTHrP signalling disorders (iPPSD)

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Abstract

Albright Hereditary Osteodystrophy (AHO) is a complex disorder defined by the presence of a short adult stature relative to the height of an unaffected parent and brachydactyly type E, as well as a stocky build, round face, and ectopic calcifications. AHO and pseudohypoparathyroidism (PHP) have been used interchangeably in the past. The term PHP describes end-organ resistance to parathyroid hormone (PTH), occurring with or without the physical features of AHO. Conversely, pseudopseudohypoparathyroidism (PPHP) describes individuals with AHO features in the absence of PTH resistance. PHP and PPHP are aetiologically linked and caused by genetic and/or epigenetic alterations in the guanine nucleotide-binding protein alpha-stimulating ($G_s\alpha$) locus (*GNAS*) in chromosome 20q13. Another less-recognised group of skeletal dysplasias, termed acrodysostosis, partially overlap with skeletal, endocrine, and neurodevelopmental features of AHO/PHP and can be overlooked in clinical practice, causing confusion in the literature. Acrodysostosis is caused by defects in two genes, *PRKAR1A* and *PDE4D*, both encoding important components of the $G_s\alpha$- cyclic adenosine monophosphate (cAMP)-protein kinase A (PKA) signalling pathway. We describe the clinical course and genotype of two adult patients with overlapping AHO features who harboured novel pathogenic variants in *GNAS* (c.2273C>G, p.Pro758Arg, NM_080425.2) and *PRKAR1A* (c.803C>T, p.Ala268Val, NM_002734.4), respectively. We highlight the value of expert radiological opinion and molecular testing in establishing correct diagnoses and discuss phenotypic features of our patients, including the first description of subcutaneous ossification and spina bifida occulta in *PRKAR1A*-related acrodysostosis, in the context of the novel inactivating PTH/PTH related peptide (PTHrP) signalling disorder (iPPSD) classification system.

Key Words

Acrodysostosis, pseudohypoparathyroidism, pseudopseudohypoparathyroidism, Albright Hereditary Osteodystrophy, iPPSD

Introduction

Albright Hereditary Osteodystrophy (AHO; OMIM #103580), first described in 1942 by Albright and colleagues, is a syndrome characterised by short adult stature, round face, brachydactyly, a stocky habitus and ectopic calcifications (Mantovani et al., 2018)(Mantovani, Spada, & Elli, 2016). Parathyroid hormone (PTH) resistance was initially considered an obligatory manifestation of AHO with the terms AHO and pseudohypoparathyroidism (PHP) being used interchangeably. However, it was later recognised that AHO can occur in the absence of PTH resistance, described as pseudopseudohypoparathyroidism (PPHP; OMIM #612463). Both PHP and PPHP result from genetic and/or epigenetic alterations at the guanine nucleotide-binding protein alpha-stimulating ($G_s\alpha$) locus (*GNAS*; OMIM*139320) subject to imprinting. The $G_s\alpha$ protein is an essential component of the cyclic adenosine monophosphate (cAMP) signalling pathway mediating physiological responses to hormones that bind G-protein-coupled receptors (GPCR), including PTH (Figure 1). The resulting cAMP acts as a secondary messenger interacting with and regulating other proteins, including protein kinase A (PKA), with phosphorylation of downstream targets.

There are several forms of PHP, all sharing biochemical features of end-organ resistance to the action of PTH (elevated PTH levels which in some cases are associated with hypocalcaemia and hyperphosphataemia in the absence of vitamin D deficiency) (Mantovani et al., 2018). PHP type I is characterised by a blunted cAMP response to exogenous PTH whereas in PHP type II (PHP2; OMIM #203330), increased cAMP is observed but with a deficient phosphaturic response. PHP type I is further subclassified into PHPIa (OMIM #103580) and PHPIc (OMIM #612462) with concomitant AHO features and PHPIb (OMIM #603233) generally without AHO. The phenotypes and underlying molecular mechanisms of PHP and PPHP, including other *GNAS*-related disorders such as progressive osseous heteroplasia (OMIM #166350), are summarised in Figure 1.

The AHO phenotype is not unique to the above conditions and can lead to an incorrectly assigned clinical diagnosis of PHP in individuals who tested negative for *GNAS* molecular defects (Francesca Marta Elli et al., 2016). A common source of confusion in prior literature is that of acrodysostosis, otherwise known as

Maroteaux-Malamut syndrome, initially described in the 1970s as a clinical and radiological entity (Maroteaux & Malamut, 1968; Silve et al., 2012). Acrodysostosis is defined as 'the association of severe brachydactyly, facial dysostosis and nasal hypoplasia' (Mantovani et al., 2018). The bony abnormalities of acrodysostosis are often indistinguishable from AHO. (Mantovani et al., 2016). Other observed features include advanced bone age, developmental delay, obesity, hearing loss and progressive growth failure with short stature. On biochemical testing, resistance to multiple hormones, including PTH, thyroid stimulating hormone (TSH) and the gonadotropins, that utilise the cAMP signalling pathway can be detected in a proportion of affected individuals. The condition is sub-classified into acrodysostosis types 1 (OMIM #101800) and 2 (OMIM #614613), involving *PRKAR1A* (OMIM *188830) and *PDE4D* (OMIM *600129) genes respectively, encoding for key players of the GPCR- $G_s\alpha$-cAMP-PKA pathway (Figure 1).

Overall, the above disorders result from an impairment in the PTH-receptor signalling pathway at the molecular level with some overlapping clinical and biochemical features. Past classification systems for PHP have failed to include the underlying molecular defect of PHP subtypes and have not been inclusive of their phenocopies, such as progressive osseous heteroplasia and acrodysostosis. The EuroPHP network thus suggested a new classification system under the term inactivating PTH/PTHrP signalling disorders (iPPSD) (Thiele et al., 2016). This allows for a greater appreciation of the phenotypic and molecular parallels between disorders of the PTH-receptor's GPCR- $G_s\alpha$-cAMP-PKA pathway and the diagnostic challenge they can represent.

This report describes two adult individuals with overlapping clinical features of AHO who carry heterozygous novel pathogenic variants in the *GNAS* and *PRKAR1A* genes, respectively. Their phenotypes are described in detail, with the first description of subcutaneous ossification and spina bifida occulta in a patient with *PRKAR1A*-releated acrodysostosis, which we believe helps expand the phenotypic spectrum of PHP and acrodysostosis type 1, or iPPSD2 (involving *GNAS* mutations) and iPPSD4 (involving *PRKAR1A* mutations), respectively, as defined in the EuroPHP-proposed novel classification (Thiele et al., 2016). Our cases not only illustrate the value of an expert radiological opinion and application of Next Generation Sequencing (NGS) panel gene testing in achieving a molecular diagnosis, but also highlight the diagnostic journey of these patients alongside the recommendations from the recently published consensus statement on PHP and its related disorders, to better illustrate the benefits of this guidance (Mantovani et al., 2018).

Clinical report

Patient 1

Patient 1 was a 58-year-old male and the third child of non-consanguineous parents of white British origin. He was born at term by spontaneous vaginal delivery after an uneventful pregnancy with no concerns antenatally or postnatally. He reached his early milestones at an appropriate age with no other developmental concerns and no visual or auditory problems. He had poor engagement with education throughout childhood (self-reported), and left education at 17 years of age without any qualifications; he was later diagnosed with mild learning difficulties. As an adult, he is able to live independently and earns a living working in his local supermarket. He is not in a relationship and has no children. Past medical history in chronological order included: gout as a young adult, and hypertension, with an onset at the age of 45 years. He was diagnosed with sarcoidosis at 46 years of age, having presented with cutaneous, renal, and pulmonary manifestations of the disease. Following prolonged corticosteroid treatment necessary for his diagnosis of sarcoidosis, he later developed corticosteroid-induced diabetes mellitus at 51 years of age, presenting with marked hyperglycaemia requiring hospitalisation and treated with insulin. At that time, he was documented clinically and radiologically to have brachydactyly. Complications of his sarcoidosis include stage III chronic kidney disease, pulmonary emphysema, and a single episode of a secondary spontaneous pneumothorax at 53 years of age. Additional past medical history included ischaemic heart disease that required a percutaneous coronary intervention at 55 years of age, and secondary hypoadrenalism at 57 years of age because of exogenous chronic glucocorticoid use.

He was referred from the diabetes clinic (Dr Amanda Adler) to the Cambridge Clinical Genetics department at 57 years of age to investigate his longstanding biochemical and skeletal features as a genetic syndrome was suspected. These included a persistently raised serum PTH without an affected correct serum calcium level, shortened metacarpals and metatarsals, and a general Cushingoid appearance. Whilst the first can be attributed to his chronic kidney disease, and the last to his long-term steroid treatment, his skeletal features, in his hands and feet, could not be attributed to any aspect of his past medical history. There was no family history of endocrinopathy, skeletal dysplasia, or learning difficulties.

On first presentation he was noted to have disproportionate short stature [(height of 158.4 cm; < 0.4th centile) (Freeman et al., 1995)] with relatively short limbs (Figure 2). His occipitofrontal circumference was 58cm (75th - 90th centile) (Bushby, Cole, Matthews, & Goodship, 1992)] showing relative macrocephaly. His weight was 69.6kg with central obesity (body mass index of 27.7 kg/m^2). He had small palpebral fissures, thin lips, and a normal palate. There was bilateral brachydactyly, with short 4th and 5th metacarpals and metatarsals, and short terminal phalanges of the thumbs. He had multiple warts on his hands and forearms, but no cutaneous manifestations of sarcoidosis. Mild pectus excavatum was noted.

Biochemical investigations showed persistently elevated serum PTH levels of 13.36-17.07pmol/l) (normal range 1.48 - 7.63); normal corrected serum calcium levels (normal range 2.2 - 2.6), aside from a single elevated level of 2.64 mmol/L (normal range 2.2 - 2.6); and consistently normal serum phosphate levels (normal range 0.8 - 1.5). Serum TSH levels were normal at 3.57 mU/L and 3.11 mU/L (normal range 0.35 - 5.5), and plasma vitamin D levels were normal. Renal function and blood electrolyte levels were consistent with stage III chronic kidney disease, and serum angiotensin-converting enzyme levels fluctuated with the course of the sarcoidosis.

Patient 2

Patient 2 was a 47-year-old male, the first child of non-consanguineous parents of white South African origin. At the time of his birth, his mother and father were aged 20 and 27 years respectively. Intrauterine growth restriction was noted antenatally, and he was born at 42 weeks of gestation by spontaneous vaginal delivery after induction of labour. Birth weight was 2.2kg [(<0.4th centile) (Freeman et al., 1995)]. Resuscitation was needed at birth, and he spent 5 days in the Special Care Baby Unit. Despite initial issues with breastfeeding and milk intolerance, once he started bottle feeding, he achieved satisfactory growth. Motor milestones were met normally, with walking by 1 year of age, but speech delay was noted in early childhood; following investigation, conductive hearing loss was detected at 2 years of age. Ear, nose and throat conditions persisted throughout childhood, including 12 sets of grommets, mastoidectomy following two episodes of cholesteatoma, bilateral hearing aids, and recurrent sinus problems. He also needed speech and language therapy for many years with concurrent mild learning difficulties, for which he attended a special needs school in South Africa. He left education at 17 years and attended night school to obtain further qualifications. He is able to live independently.

He was investigated throughout childhood in South Africa for his unusual past medical history, particularly his short stature, and was diagnosed with AHO at 14 years of age in Durban, South Africa. He was also investigated for potential sarcoidosis of the lungs in Oxford, UK at 43 years of age and now has a diagnosis of chronic obstructive pulmonary disease and suffers from a chronic cough. Other history of note included a basal cell carcinoma removed from his right leg, L4/5 laminectomy, spinal cyst removal, and gastro-oesophageal reflux disease. Noting a prior diagnosis of AHO, his general practitioner referred him to Dr Kenneth Poole at Addenbrooke's Hospital Rare Bone disease clinic (part of ERBON, the East Anglian Rare BONe disease network) for management. He was subsequently referred to the clinical genetics department at 44 years for a genetic assessment.

On first presentation, he was noted to have disproportionate short stature (height of 163cm; 0.4th - 2nd centile) (Freeman et al., 1995)] with relatively long trunk and short limbs (Figure 2). His occipitofrontal circumference was 58cm [(75th - 90th centile) (Bushby et al., 1992)] showing a relative macrocephaly. Weight was 83kg with centripetal obesity (body mass index of 31.2). He had round facies with full cheeks, short nose with broad nasal bridge, thin upper lip, and a high palate. On peripheral limb examination, there was marked brachydactyly in his hands and feet with particularly short distal phalanges and metacarpals and metatarsals (Figure 2). He had a 1cm diameter area of gritty calcification over the lateral side of the dorsum of his proximal right hallux.

Biochemical investigations were suggestive of resistance to PTH, with sequential raised serum PTH of 37.84 pmol/L and 21.94 pmol/L (normal range 1.48 - 7.63), normal corrected serum calcium of 2.4 mmol/L and 2.32 mmol/L (normal range 2.2 - 2.6) and serum phosphate of 1.05 mmol/L and 0.98 mmol/L (normal range 0.8 - 1.5). He was vitamin D replete. No other hormone abnormalities were detected apart from marginally elevated serum luteinising hormone (LH) on a single occasion: sequential serum TSH of 4.16 mU/L and 5.38 mU/L (normal range 0.35 - 5.5), LH of 7.0 U/L and 5.1 U/L (normal range 1.5 - 6.3), and FSH of 6.0 U/L and 6.6 U/L (normal range 1-10). Kidney function and other serum electrolytes were all within normal range.

Methods

Chromosome micro-array analysis was performed using the Affymetrix CytoScan 750k SNP genotyping array. The analysis was performed at a genome-wide resolution of 200kb using Affymetrix Chromosome Analysis Suite (ChAS) software (Build 37). Next Generation Sequencing (NGS) of the coding regions (+/-5 bp) of the

PDE4D, *PRKAR1A*, and *GNAS* genes using the Illumina TruSight One sequencing panel, using an in-house bioinformatics pipeline. Whole exon deletions/duplications and other large rearrangements are not detected with this method. 100% target sequence coverage to a read depth of 20-fold or more was achieved for the *GNAS* and *PRKAR1A* genes, and over 97% for the *PDE4D* gene. Variant interpretation was performed following ACMG standards and guidelines for variant interpretation (Richards et al., 2015). Any pathogenic or likely pathogenic sequence changes detected by NGS were confirmed by Sanger sequencing.

Results

Chromosome microarray analysis was performed in both individuals in view of the history of mild learning difficulties with no clinically significant abnormalities in either, including absence of chromosome 2q37 deletion which can cause an AHO-like phenotype with brachydactyly and intellectual disability (Wilson et al., 1995). Sequencing of *PDE4D*, *PRKAR1A and GNAS* in patient 1 revealed a likely pathogenic heterozygous sequence change in *GNAS* (c.2273C>G, p.Pro758Arg, NM_080425.2). This variant was not previously reported in population frequency databases (Genome Aggregation Database, Exome Variant Server, 1000 Genome) and affects a highly conserved amino acid in a part of the gene that is relative intolerant to missense variation. In silico analyses predict a deleterious effect or probably damaging using SIFT and PolyPhen, respectively. Other missense changes at the same amino acid residue have been previously reported as pathogenic changes associated with AHO as part of either PHPIa or PPHP (Ahmed et al., 1998; Ahrens et al., 2001). Due to the concurrent chronic kidney disease, it is difficult to assess whether the patient's raised PTH, is due to the reduced kidney function or the above mutation, and hence it is difficult to assign this novel pathogenic variant to either PHPIa or PPHP. Cascade family testing has been declined.

In Patient 2, targeted sequencing of the *GNAS* gene did not identify any pathogenic variants. A skeletal survey was then performed upon the individual's wishes to further delineate his phenotype. Overall, the radiographic findings reported by the UK skeletal dysplasia expert radiologist Dr Amaka Offiah (Figure 2) were phenotypically consistent with PHP, PPHP and acrodysostosis. He was also noted to have spina bifida occulta of the lower lumbar spine. Given the negative *GNAS* mutation analysis, sequencing of the *PDE4D* and *PRKAR1A* genes was undertaken for suspected acrodysostosis with an identified likely pathogenic heterozygous sequence change (c.803C>T, p.Ala268Val, NM_002734.3) in the *PRKAR1A* gene. This variant has not previously been reported in population databases. *In silico* analysis predicts that this highly conserved residue is very likely to be damaging and lies in the cAMP-binding domain B where other pathogenic variants have been reported to be associated with acrodysostosis (Linglart et al., 2012). Subsequent parental testing confirmed *de novo* occurrence.

Discussion

In the first case study, our patient exhibited the AHO phenotype and elevated PTH levels with a novel *GNAS* mutation, which was predicted to be likely pathogenic. It is difficult to discern whether this patient falls under the PHPIa or PPHP category as his co-morbidity of chronic kidney disease may also be the cause of the elevated PTH due to secondary hypoparathyroidism. This highlights how endocrine co-morbidity makes characterisation of novel variants difficult in this case. Further familial testing could help resolve this issue, to shed more light on this variant. The intellectual disability usually results from maternal inheritance of the *GNAS* variant and therefore, a degree of PTH resistance is suspected in addition to the effects of chronic kidney disease on PTH.

To date, 279 *GNAS* mutations have been reported (http://www.hgmd.cf.ac.uk/ac/all.php), including predominantly protein-truncating variants (frameshift, nonsense, and splice site). These are distributed across the 13 exons of the *GNAS* gene with the exception of the known hotspot in exon 7 for the 4-bp deletion c.565_568delGACT (F. M. Elli et al., 2013). Inactivating *GNAS* disorders have complete penetrance with childhood-onset manifestations, similar to our first patient, although the number and degree of these can vary significantly between affected individuals. Overall, there does not appear to be a clear correlation between the type and location of inactivating *GNAS* pathogenic variants and clinical manifestations, with the exception of a greater prevalence of ectopic ossifications associated with protein-truncating or exon 1 variants (F. M. Elli et al., 2013; Thiele et al., 2015). Our patient harboured a missense variant in exon 5 of the *GNAS* gene with no ectopic calcifications.

Despite the high detection rate of *GNAS* molecular defects, around 30% of patients who have a clinical diagnosis of a PHP-AHO disorder still lack a molecular diagnosis (Francesca Marta Elli et al., 2016). In our second patient, it was only after initial (negative) *GNAS* testing and a skeletal survey with expert radiological opinion that further molecular testing was undertaken to reveal a diagnosis of acrodysostosis. This was difficult to discern on clinical grounds although some subtle differences exist. Brachydactyly of the hands is generally of variable degree in

PHP/PPHP as opposed to acrodysostosis, where it is more pronounced with accompanying cone-shaped epiphyses as a constant finding in the latter (Silve et al., 2012). Ectopic ossifications are not reported in association with acrodysostosis but it was detected in our patient with the *PRKAR1A* variant (Silve et al., 2012). Radiological findings consistent with acroscyphodysplasia, characterised by scypho-deformity of the knee, have been recently reported as a phenotypic variation of PHP and acrodysostosis type 2, caused by either a *GNAS* or *PDE4D* mutation, respectively (Mitsui et al., 2014). Another variable distinguishing feature is that of prenatal growth retardation, especially in acrodysostosis type 1, in contrast with normal growth pattern in the majority of PHP/PPHP cases with adult short stature preceded by a ceasing period in puberty (Silve et al., 2012). Finally, hearing loss and recurrent otitis media are commonly reported features of acrodysostosis.

Acrodysostosis type 1 involves heterozygous pathogenic variants in the *PRKAR1A* gene, which codes for the most abundantly expressed regulatory subunit of PKA, RIα. The protein consists of a dimerization domain, an inhibitory site, and two cyclic nucleotide binding domains (NBD-A and -B) where all causative variants have been detected. (Lee et al., 2012; Linglart et al., 2011, 2012; Michot et al., 2012; Muhn et al., 2013; Nagasaki et al., 2012) (Figure 1). Our second patient harboured a heterozygous missense variant in exon 9 of the *PRKAR1A* gene, overlapping with the NBD-B domain. Of the two NBDs, NBD-B is the more affected, and one of its coding exons, exon 11, contains over half of reported mutations to date (Francesca Marta Elli et al., 2016). The majority of described variants are private missense mutations (similar to our patient's) with a few functional studies indicating decreased binding affinity of cAMP to PKA by affecting the conformation of the NBDs, which in turn decreases PKA activation (Silve et al., 2012).

Of note, a series of *PRKAR1A* mutations, including single-base substitutions, small indels, and large deletions covering most of an exon, are associated with Carney complex, a rare multiple neoplasia syndrome characterised by pigmented lesions and myxomatous tumours (Correa, Salpea, & Stratakis, 2015). Overall, there is no molecular overlap between *PRKAR1A* variants in acrodysostosis and Carney complex, with the latter causing *PRKAR1A* haploinsufficiency and loss of the regulation of the catalytic subunit with increased PKA activation (Figure 1) (Rhayem et al., 2015). In addition, there are no known *PRKAR1A* genotype-phenotype correlations to date.

As illustrated by Figure 2, there is a significant phenotypic overlap between PHP and acrodysostosis. Poor differentiation due to the overlapping features between these genetically-related disorders and challenges in the recognition of these disorders by adult physicians, especially the more recently molecularly defined disorder of acrodysostosis, also contributed to a delayed time to diagnosis. Recently published classification guidelines alongside a new international consensus by the EuroPHP network are attempting to resolve this issue using the umbrella term inactivating PTH/PTH related peptide (PTHrP) signalling disorders (iPPSD), with a clinical diagnosis being aided by major and minor criteria (Mantovani et al., 2018; Thiele et al., 2016). Our two patients had overlapping major (PTH resistance, brachydactyly type E) and minor findings (cognitive impairment, obesity, abnormal facies), with respective diagnoses of iPPSD2 (due to the presence of a novel *GNAS* mutation) and iPPSD4 (due to the presence of a novel *PRKAR1A* mutation).

In conclusion, our cases demonstrate that a multi-disciplinary approach between specialists from metabolic bone disease, diabetes and endocrinology, clinical genetics, radiology and the genetics laboratory enabled the correct diagnosis to be reached where this was not possible in their childhood. Our *PRKAR1A*-related acrodysostosis patient also confirms that ectopic ossification can occur in this disorder where previously, this features was thought to be solely associated with *GNAS* pathogenic variants. He also had spina bifida occulta, a feature not previously reported, detected on his skeletal survey. The use of panel gene testing using NGS can now improve diagnostic yield. Our report also emphasises the importance of seeking the opinion of a radiologist with expertise in skeletal dysplasias as an alternative means to establishing a diagnosis.

Figure 1. *The cAMP signalling pathway, showing how the activation of a G-protein-coupled receptor causes a signalling cascade involving G-proteins, adenylyl cyclase, cAMP, and protein kinase A (downstream mediators not shown). The table below describes genetic conditions associated with defects in this pathway. Abbreviations: AC, adenylyl cyclase; AHO, Albright Hereditary Osteodystrophy; C, catalytic; GHRH, growth hormone–releasing hormone; PTH, parathyroid hormone; R, regulatory; TSH, thyroid-stimulating hormone; UPD, uniparental disomy. Table adjusted from (Mantovani et al., 2016).*

Figure 2. *(A) Patient 1, (B) Patient 2, (C) radiological features in patient 2. (C, top and middle) DP radiographs of the hands and feet show short distal phalanges, short third and fourth metacarpals of the left hand, short third, fourth and fifth metacarpals of the right hand, and a short left fourth metatarsal. (C, bottom) AP and lateral radiographs of the lumbar spine show narrow interpedicular distances with short pedicles and spina bifida occulta of the lower lumbar spine.*

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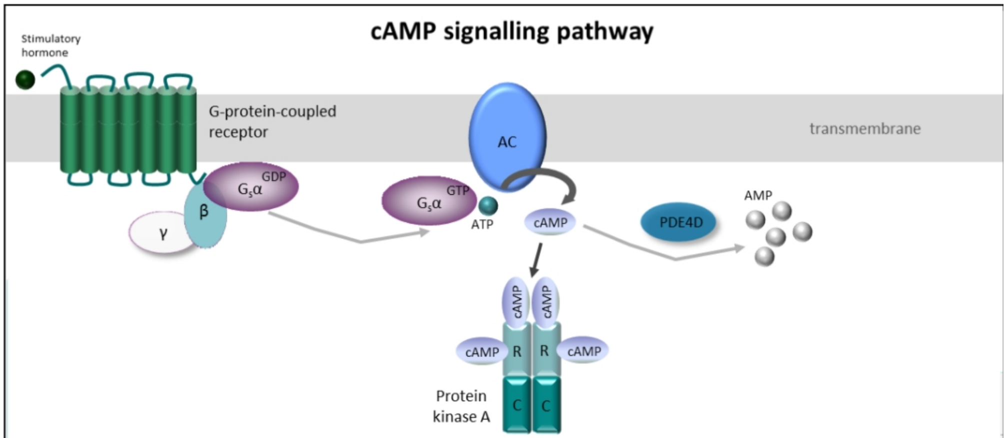

Defects in the a-stimulatory subunit of the G-protein (*GNAS*)

Condition	Phenotype	Molecular mechanism
Pseudohypoparathyroidism type Ia	• AHO • Hormonal resistance (to PTH and TSH/ GHRH/ gonadotrophins)	Maternal mutations in *GNAS*
Pseudohypoparathyroidism type Ib	• Hormonal resistance (to PTH and TSH/ GHRH/ gonadotrophins) • Rarely AHO	Deletions involving *GNAS* locus (occasionally UPD)
Pseudohypoparathyroidism type Ic	• Features of pseudohypoparathyroidism type Ia • Normal erythrocyte $G_s\alpha$ activity	Maternal mutations in *GNAS*
Pseudopseudohypopara-thyroidism	• AHO	Paternal mutations in *GNAS*
Progressive osseous heteroplasia	• Invasive heterotopic ossification	Paternal mutations in *GNAS*
McCune-Albright syndrome	• Polyostotic fibrous dysplasia • Café-au-lait skin pigmentation • Precocious puberty, hyperthyroidism, growth hormone excess, hypercortisolism	Early embryonic postzygotic somatic activating mutations in *GNAS*

Defects in cAMP-dependent protein kinase type Ia regulatory subunit (*PRKAR1A*) and cAMP-specific phosphodiesterase 4D (*PDE4D*)

Condition	Phenotype	Molecular mechanism
Acrodystostosis type 1	• AHO • Hormonal resistance (to PTH and/or TSH) in 60-70%	Mutations in *PRKAR1A* reducing affinity to cAMP
Acrodystostosis type 2	• AHO • Hormonal resistance (to PTH and/or TSH) in 10-20% • Cryptorchidism and/or lack of pubertal spurt in 19%	Mutations in *PDE4D*
Carney complex	• AHO	Mutations in *PRKAR1A* increasing affinity to cAMP

The cAMP signalling pathway, showing how the activation of a G-protein-coupled receptor causes a signalling cascade involving G-proteins, adenylyl cyclase, cAMP, and protein kinase A (downstream mediators not shown). The table below describes genetic conditions associated with defects in this pathway. Abbreviations: AC, adenylyl cyclase; AHO, Albright Hereditary Osteodystrophy; C, catalytic; GHRH, growth hormone–releasing hormone; PTH, parathyroid hormone; R, regulatory; TSH, thyroid-stimulating hormone; UPD, uniparental disomy. Table adjusted from (Mantovani et al., 2016).

205x239mm (150 x 150 DPI)

(A) Patient 1, (B) Patient 2, (C) radiological features in patient 2. (C, top and middle) DP radiographs of the hands and feet show short distal phalanges, short third and fourth metacarpals of the left hand, short third, fourth and fifth metacarpals of the right hand, and a short left fourth metatarsal. (C, bottom) AP and lateral radiographs of the lumbar spine show narrow interpedicular distances with short pedicles and spina bifida occulta of the lower lumbar spine

336x420mm (72 x 72 DPI)